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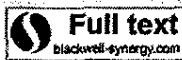
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A bifunctional epimerase-reductase acts downstream of the MUR1 gene product and completes the de novo synthesis of GDP-L-fucose in *Arabidopsis*.

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Bonin CP, Reiter WD.

University of Connecticut, Department of Molecular and Cell Biology, Storrs, CT 06269, USA.

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L-Fucose is a monosaccharide found as a component of glycoproteins and cell wall polysaccharides in higher plants. The MUR1 gene of *Arabidopsis thaliana* encodes a GDP-D-mannose 4,6-dehydratase catalyzing the first step in the de novo synthesis of GDP-L-fucose from GDP-D-mannose (Bonin et al. 1997, Proc. Natl Acad. Sci. USA, 94, 2085-2090). Plant genes encoding the subsequent steps in L-fucose synthesis (3,5-epimerization and 4-reduction) have not been described previously. Based on sequence similarities to a bacterial gene involved in capsule synthesis we have cloned a gene from *Arabidopsis*, now designated GER1, which encodes a bifunctional 3, 5-epimerase-4-reductase in L-fucose synthesis. The combined action of the MUR1 and GER1 gene products converts GDP-D-mannose to GDP-L-fucose in vitro demonstrating that this entire nucleotide-sugar interconversion pathway could be reconstituted using plant genes expressed in *Escherichia coli*. In vitro assays indicated that the GER1 protein does not act as a GDP-D-mannose 3, 5-epimerase, an enzymatic activity involved in the de novo synthesis of GDP-L-galactose and L-ascorbic acid. Similarly, L-ascorbate levels in GER1 antisense plants were unchanged indicating that GDP-D-mannose 3,5-epimerase is encoded by a separate gene.

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An epimerase-reductase in L-fucose synthesis.

Chang S, Duerr B, Serif G.

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Department of Biochemistry, Ohio State University, Columbus 43210-1292.

The first committed enzyme in GDP-L-fucose formation from GDP-D-mannose is GDP-D-mannose 4,6-dehydratase, which forms GDP-4-keto-6-deoxy-D-mannose. The uncertain enzymatic steps beyond this point were examined in this study. Assays were developed for the epimerase and reductase activities which the putative pathway would predict. A protein was isolated exhibiting homogeneity by several criteria. This single protein, which forms GDP-L-fucose from GDP-4-keto-6-deoxy-D-mannose and NADH, appears to possess both epimerase and reductase capabilities and may be termed GDP-4-keto-6-deoxy-D-mannose-3,5-epimerase-4-reductase. Analysis on a molecular sieve column using fast protein liquid chromatography established a molecular weight of 63,100 for the native enzyme, whereas sodium dodecyl sulfate-polyacrylamide gel electrophoresis established a subunit molecular weight of 31,500.

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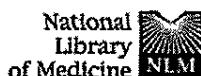
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Probing the catalytic mechanism of GDP-4-keto-6-deoxy-d-mannose Epimerase/Reductase by kinetic and crystallographic characterization of site-specific mutants.

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Services**Rosano C, Bisso A, Izzo G, Tonetti M, Sturla L, De Flora A, Bolognesi M.**Department of Physics-INFM and Advanced Biotechnology Center-IST,
University of Genova, Largo Rosanna Benzi 10, Genova, I-16132, Italy.Related
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GDP-4-keto-6-deoxy-d-mannose epimerase/reductase is a bifunctional enzyme responsible for the last step in the biosynthesis of GDP-1-fucose, the substrate of fucosyl transferases. Several cell-surface antigens, including the leukocyte Lewis system and cell-surface antigens in pathogenic bacteria, depend on the availability of GDP-1-fucose for their expression. Therefore, the enzyme is a potential target for therapy in pathological states depending on selectin-mediated cell-to-cell interactions. Previous crystallographic investigations have shown that GDP-4-keto-6-deoxy-d-mannose epimerase/reductase belongs to the short-chain dehydrogenase/reductase protein homology family. The enzyme active-site region is at the interface of an N-terminal NADPH-binding domain and a C-terminal domain, held to bind the substrate. The design, expression and functional characterization of seven site-specific mutant forms of GDP-4-keto-6-deoxy-d-mannose epimerase/reductase are reported here. In parallel, the crystal structures of the native holoenzyme and of three mutants (Ser107Ala, Tyr136Glu and Lys140Arg) have been investigated and refined at 1.45-1.60 Å resolution, based on synchrotron data (R-factors range between 12.6 % and 13.9 %). The refined protein models show that besides the active-site residues Ser107, Tyr136 and Lys140, whose mutations impair the overall enzymatic activity and may affect the coenzyme binding mode, side-chains capable of proton exchange, located around the expected substrate (GDP-4-keto-6-deoxy-d-mannose) binding pocket, are selectively required during the epimerization and reduction steps. Among these, Cys109 and His179 may play a primary role in proton exchange between the enzyme and the epimerization catalytic intermediates. Finally, the additional role of mutated active-site residues involved in substrate recognition and in enzyme stability has been analyzed.

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